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Notes on the Use of Clove Oil As An Anaesthetic for Northern Fish Species And for the Surgical Implantation of Radio Transmitters Into Arctic Grayling (Thymallus Arcticus)

B. G. Blackman
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Peace/Williston Fish and Wildlife Compensation Program, 1011 Fourth Ave. 3rd Floor, Prince George B.C. V2L 3H9

Website: *www.bchydro.bc.ca/environment/initiatives/pwcp/*

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Author(s): Brian G. Blackman[1]

Address(es): [1] Peace/Williston Fish and Wildlife Compensation Program, 1011 Fourth Ave., 3rd Floor Prince George, B.C. V2L 3H9

ABSTRACT

MS-222 or 3-aminobenzoic acid ethyl - ester methanosulfate is an effective anaesthetic for most fish species. However, in many areas the use of this drug requires that fish be held for an extended period of time before release into the wild, making this anaesthetic impractical for most field applications. Clove oil, has recently been proposed as an alternative anaesthetic. It is used throughout the world as food flavouring and as a local anaesthetic in dentistry. Eugenol (4-allyl-2-methoxy-phenol), the active ingredient in clove oil, has also been reported to have anti-bacterial, antifungal and anti-oxidant capabilities. Clove oil was "field tested" for use as a general anaesthetic for a number of local freshwater fish species and as an anaesthetic for the surgical implantation of radio transmitters into Arctic grayling *(Thymallus arcticus)*. After initial tests, clove oil at a concentration of 40 mg/L was used to surgically implant radio transmitters into 17 Arctic grayling. Mean time to achieve level 5 anaesthesia was 178 seconds $\pm$23 seconds, average surgery time was 240 seconds $\pm$ 34 seconds and average recovery time was 287 seconds $\pm$ 51 seconds. Based in these initial tests, clove oil appears to be a suitable alternative anaesthetic.

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INTRODUCTION

MS-222 or 3-aminobenzoic acid ethyl - ester methanosulfate is an effective anaesthetic for most fish species. However, the use of this drug requires that fish be held for a minimum of 21 days before release or consumption by humans, (US food and Drug Administration) making it impractical for most field applications.

An alternative anaesthetic is being tested (Anderson et al 1997) that appears to be a suitable substitute. Clove oil is used throughout the world as food flavouring and has an accepted human daily intake of 2.5 mg/kg (World Health Organization). It is also used as a local anaesthesia in dentistry. Eugenol (4-allyl-2-methoxy-phenol), the active ingredient in clove oil has anaesthetic properties (Anderson et al 1997) and in addition, is reported to have anti-bacterial activity, (Karapmar and Aktug 1987: Briozzo, et al., 1989), antifungal activity (Bullerman et al.1977) and anti-oxidant capabilities (Cort, 1974: Rajakumarand Rao, 1993).

The intent of the project was to test clove oil as an anaesthetic on several local fish species and to surgically implant radio transmitters into adult Arctic grayling *(Thymallus arcticus)* using clove oil as an anaesthetic. This report is a collection of observations made during the process to help provide additional information on the use of clove oil as an anaesthetic.

METHODS

The use of clove oil as an anaesthetic was evaluated in two phases. Phase one consisted of evaluating the most suitable concentrations with simulated surgery, practice surgery (incisions and sutures but no tags) and recovery times. Phase two consisted of recording data from surgical implants. Radio transmitters were implanted into 55 Arctic grayling, however the times required to attain the different levels of anaesthesia, for surgery, and recovery were only recorded for the last 17 fish.

Initial tests were conducted on fish that were captured by beach seine or angling. Fish were held in plastic coolers filled with river water or in recovery tubes placed in the river. Water was changed frequently in the coolers to avoid stress caused by lack of oxygen. No fish that were surgically implanted with transmitters were held for more than 20 minutes prior to surgery.

Fish were weighed using an electronic balance the nearest 10 g. Fish that were too large for the balance (>400 g) were weighed to the nearest ounce using an electronic scale (Normark 50 LB. electronic scale) and the data converted to grams.

Clove oil does not mix well with cold water so a 1:10 clove oil ethanol solution was used and 4.4 ml of the solution were added to 10 litres of river water to give a 40 ppm solution of anaesthetic. The solution was prepared fresh each day to reduce oxidation of the clove oil.

Fish were placed in the anaesthetic and their reactions observed and timed. Times were recorded to the different stages of anaesthesia using the following criteria (from McKinley et al 1996, modified as indicated).

Stage	Behaviour
1	Slight loss of reactivity to external stimuli, equilibrium normal
2	Loss of reactivity to external stimuli, equilibrium normal
3	Increased opercular rate, equilibrium waning
4	Slow but regular opercular rate, equilibrium lost
5	Opercular movements slow and irregular
6	Opercular movements cease, cardiac arrest.

Stage 3* was recorded as the time taken to when the fish first lost its upright position.
Stage 5* was achieved when the fish no longer responded to a sharp pinch on the adipose fin.

During surgery a thin strip of foam rubber was placed under the fish to keep the under side of the fish moist.

A ten litre plastic water bottle equipped with a shut off valve and 1 meter of surgical tubing was used to provide an anaesthetic drip during surgery. A low concentration (5-10 ppm) of anaesthetic was washed over the gills of the fish during the surgery. Different concentrations of the dripped solution were tested to in ensure the fish remained under the effect of the anaesthetic, but did not have longer recovery times.

Surgery consisted of making a 3 centimetre incision 3-4 centimetres from the mid ventral line, anterior to the pelvic fins. Then a hollow needle was inserted through the body wall, posterior to the incision and the transmitter antenna was threaded out through the centre of the needle. The transmitter (10.9 x43 mm , 7.7 g) was inserted into the body cavity and pulled backward by the antenna. The incision was then closed using three to four permanent independent sutures. Surgical instruments were washed with alcohol between implants to provide a more sterile procedure.

All fish were measured, weighed and scale and tissue samples (adipose fin for genetic analysis) were collected.

After surgery was completed the fish were placed in the river to revive in temporary pens constructed from river rocks. The fish were held upright and gently moved back and forth to increase water flow over the gills. When they were able to maintain an upright position without assistance, the fish were deemed to have recovered. The fish remained in the pens until they swam out on their own.

Only three fish were tagged at each location so fresh anaesthetic was used for all implants, but frequently up to 20 other fish were captured and sampled at a site.

The implant surgery was carried out during the period from August 22, to September 6 1996 in the Table and Anzac Rivers. Water temperatures during this period were approximately 8-10° C

Telemetry surveys from a helicopter and on foot were carried out to determine fish movements and underwater snorkel surveys were made to determine the condition of the fish during a period from 13 to 30 days after release.

RESULTS AND DISCUSSION

It was concluded from the initial evaluations of the anaesthetic that the 40 ppm concentration of clove oil brought the fish to a stage 5 level of anaesthesia in approximately three minutes with recovery times in the five minute range. (Table 1). The drip anaesthetic with a concentration of 9 ppm (1cc/10 L) increased the recovery time as did 6 ppm (0.7cc/10 L) but 4.5 ppm (0.5 cc/10L) did not increase recovery times (Table 1). During the first few surgical implants, fish showed signs of recovery during the surgical procedure, using a drip concentration of 4.5 ppm . The drip concentration was increase to 6 ppm, which eliminated the problem.

The time required for fish to succumb to the anaesthetic ranged from 180 to 300 seconds. It was difficult to determine when the fish were at stage five of anaesthesia. Fish that showed no response to a sharp pinch on the adipose fin were considered at stage five. Prior to surgery a portion of the adipose fin was cut off with scissors and preserved as a tissue sample for genetic analysis. If the fish did not react to this, surgery proceeded. If the fish did react, they were returned to the anaesthetic for an additional 30-60 seconds.

Although no detailed notes were taken, it was observed that fish held in a recovery tube, or cooler for 5 to 10 minutes, recovered from surgery in a shorter period of time than fish that were placed in the anaesthetic immediately after capture.

Underwater snorkel surveys were made to determine the condition of the fish during a period from 13 to 30 days after release, but water conditions had deteriorated and many of the fish had started moving downstream towards deeper overwintering areas. Only two of the tagged fish, which had remained in a shallow headwater area were observed and these fish showed no ill effects from the surgery.

Table 1. Induction and recovery times from anaesthesia

Species	Weight	Anaesthesia Time Stage 3	Stage 5		Surgery Time	Anaesthetic Drip (ppm)	Recovery Time
	(g)	(s)	(s)		(s)	Concentration	(s)
MW	240	105	180		180	0	300
MW	260	230	270		180	0	330
MW	175	180	225		300	9	480
MW	250	140	180		300	9	540
MW	210	120	180	1*	300	4.5	260
MW	260	120	225		300	6	380
GR	268	110	180		300	4.5	240
GR	300		180		300	4.5	300
BT	430		180		300	4.5	300
RB	310		180		300	4.5	300
GR	270		180		300	4.5	300
MW	220		180	2*	540	4.5	1020
MW	375		300		480	4.5	900
GR	345		180		300	4.5	300
MW	280		220		360	4.5	360
MW	250		295		300	4.5	540
MW	250		220		300	4.5	~ 500

Species MW mountain whitefish (Prosopium williamsoni), GR Arctic qraylinq, BT Bull trout (Salvelinus confluentus), RB Rainbow trout (Oncorhynchus mykiss).
All times are given in seconds Water temperature was 10 ° C
Concentration of the anaesthetic was 40 ppm

*1 fish started to recover @ 260 sec in surgery phase
*2 practice surgery - incision and sutures but no transmitters, some physical response during surgery

Comments
for the first 11 fish surgery was simply holding the fish for the allotted time; for the last 6 fish included incisions and sutures

The time required to reach stage five averaged 178 seconds and ranged from 106 to 300 seconds (Table 2) so exposure time should not be used to determine stage. The average recovery time was 287 seconds and ranged from 118 to 537 seconds. These times are similar to the three and five minutes noted as "desirable" for induction and recovery from anaesthesia by Marking and Meyer (1985). These results are similar to those reported by Prince and Powell (2000) for rainbow trout *(Oncorhynchus mykiss)*. They used a concentration of 30 mg/L and had an average induction time of 222 seconds and an average recovery time of 294 seconds.

During later studies the anaesthetic mixture appeared to irritate the gills of juvenile Arctic grayling and bull trout. This was evident by a great deal of coughing, gapping and head shaking. Clove oil will oxidise and should be kept stored in a dark bottle in a cold dark place. The oxidised clove oil did not appear to have any harmful side effects. Two young of the year Arctic grayling were held in an aquarium for several months after exposure to the "oxidised" clove oil and showed no harmful side effects. If the clove oil is too oxidised or used in too high a concentration fish will exhibit this coughing and head shaking reaction and the affects can be lethal (pers comm Dr. S. McKinley, University of Waterloo).

Table 2. Arctic Grayling induction, surgical, and recovery times.

Fish No.	Weight (g)	Tag % Weight	Cumulative time in seconds to: Stage 3	Stage 5	Surgery	Sample	Recovery	Surgery Time (s)	Recovery Time(s)
39	378	1.85	53	132	348	418	611	216	193
40	385	1.82	78	151	416	437	555	265	118
41	610	1.15	84	181	443	466	1003	262	537
42	561	1.25	89	180	438	483	803	258	320
43	366	191	120	170	397	400	671	227	271
44	421	1.66	70	200	392	456	720	192	264
*45	504	1.39	80	180	630	660	900	450	240
**46	453	1.55	80	299	618	645	1010	319	365
47	329	2.13	115	253	511	541	783	258	242
48	368	1.9	80	155	410	440	650	255	210
***49	467	1.5	85	200	390	420	850	190	430
50	448	1.56	57	168	358	395	585	190	190
51	447	1.57	80	164	354	386	600	190	214
52	424	1.65	38	200	353	386	720	153	334
53	415	1.69	52	106	303	331	638	197	307
54	428	1.64	63	147	363	391	722	216	331
55	504	1.39	50	138	374	414	720	236	306
mean	441.5	1.62	74.9	177.88	417.5	451.1	737.7	239.6	286.6
min	328	1.15	38	106	303	331	555	153	118
max.	610	2.13	120	299	630	660	1010	450	537
std	72.5	0.0025	21.8	45.6	90.7	88.9	138	67.5	99.4

* fish started to recover from anaesthetic during surgery
** fish was placed back into anaesthetic for 60 sec after preparation for surgery
*** recaptured by angling (dry fly) 30 min after release.

Comments
Anaesthetic concentrations were 40 ppm
Drip Concentrations were 6 ppm
Water temperatures were 8-10 ° C

CONCLUSIONS

There did not appear to be any obvious difference in the induction and recovery times between the species tested (mountain whitefish, Arctic grayling, rainbow trout, and bull trout).

The fish were sufficiently sedated for normal sampling (length, weight, scale sample) in slightly over one minute, with an anaesthetic concentration of 40 ppm and water temperatures of 8-10 ° C. However, for surgical procedures the fish required approximately three minutes in the anaesthetic. Physiological response to pain, (a sharp pinch) appeared to be the most effective method for determining the state of anaesthesia rather than the time in, or concentration of the anaesthetic.

Most of the drugs (antibiotics, fungicides, anaesthetics etc) historically used on fish are now classified as unapproved (in Canada) and may not be used, or require approval by the Bureau of

Veterinary drugs with extensive holding periods before fish can be released. These restrictions make working with fish to be released into the wild most difficult. Clove oil, or its active ingredient eugenol, appears to potentially solve a number of problems. It is an inexpensive (~$60/kg) and effective anaesthetic. It should not require a withdrawal period, since it has an accepted human daily intake of 2.5 mg / km (World Health Organisation). Eugenol is also reported to have anti-bacterial activity, (Karapamar and Aktug 1987: Briozzo, et al., 1989), antifungal activity (Bullerman et al, 1977) and anti-oxidant capabilities (Cort, 1974: Rajakumar and Rao, 1993). It would be valuable to have studies conducted under controlled conditions to determine the anti fungal and anti bacterial properties of clove oil on fish.

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